Electronic supplementary material

Methods

Plasmids, cell culture and reporter assays All recombinant DNA work was performed according to standard procedures [1]. The Y151C mutation was introduced into the pCDNA3-PPARγ1 and pCDNA3-PPARγ2 constructs (kind gift from V. K. K. Chatterjee, University of Cambridge, Cambridge, UK) using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and verified by sequencing. The 3× PPRE-Tk-Luc reporter (kind gift from R. M. Evans, The Salk Institute for Biological Studies, La Jolla, CA, USA) and FABP4-Luc reporter (kind gift from S. Mandrup, University of Southern Denmark, Odense, Denmark) have been described by van Beekum et al. [1]. Reporter assays in human U2OS osteosarcoma cells were performed exactly as described [1–3]. Cells were seeded in 24-well plates and transiently transfected using the calcium-phosphate precipitation method. Each well was cotransfected with a reporter construct, PPAR expression constructs and 2 ng pCMV-Renilla (Promega Madison, WI, USA), as indicated in the figure legends. After washing, cells were maintained in medium in the presence or absence of rosiglitazone for 24 h. Activities of luciferase plus Renilla were measured with the dual luciferase reporter assay system (Promega, Madison, WI, USA), using a 96-well luminometer (Berthold Technologies, Bad Wildbad, Germany).

Electrophoretic mobility shift assay (EMSA) These experiments were performed as described by Jeninga et al. and Monajemi et al. [2,3]. In short, a radiolabelled double-stranded DNA oligomer, containing the PPRE from the rat acyltransferase-coenzyme A oxidase promoter, was incubated with in vitro translated PPARγ (wild-type [WT] or mutant) and/or in vitro translated RXRα proteins. Receptor–DNA complexes were separated from unbound DNA on native gels and visualised by autoradiography. The complete probe sequences used for binding and competition analysis were as follows: *PPRE* WT, 5'-CCG GGG ACC AGG

ACA AAG GTC ACG A-3', and *PPRE* mutant, 5'-CCG GGG GAC CAG CAC AAA GCA CAC GA-3'.

Results

Clinical features of the participants with Y151C mutation The index patient was a 61-year-old woman of Dutch ancestry who had participated in the Olympics as an athlete. Menarche had occurred at age 14 and was followed by regular menstrual cycles. She gave birth to a healthy son and daughter at the ages of 23 and 28, respectively. At the age of 43 years she was diagnosed with hypertension and hypertriacylglycerolaemia with eruptive xanthomas. At the age of 49 she underwent a partial pancreatectomy following an episode of necrotising pancreatitis. Shortly thereafter she developed diabetes mellitus, which was originally attributed to insulin deficiency after a pancreatectomy. At the age of 60 years she underwent endovascular stent graft placement because of a significant atherosclerotic stenosis in the iliac tract. At the age of 57 the proband was referred to our lipid clinic for management of uncontrolled hypertriacylglycerolaemia despite a combination of fibrate and statin therapy. On physical examination she was lean (BMI 22 kg/m^2) with a blood pressure of 145/70 mmHg. Laboratory analysis after an overnight fast showed a total cholesterol of 3.25 mmol/l, triacylglycerols of 36.7 mmol/l, HDL-cholesterol of 0.7 mmol/l and apolipoprotein B levels of 0.84 mmol/l, consistent with severe chylomicronaemia. Post-heparin testing for lipoprotein lipase (LPL) activity showed normal LPL activity and no genomic DNA-sequence changes were seen in the *LPL* gene. Glycated haemoglobin was 8.9 % and insulin levels were elevated, indicating insulin resistance as the primary cause of the presence of diabetes mellitus. The start of multiple daily injections of insulin totaling 140 U/day resulted in improved glycaemic control. On physical examination there was a clear excess of subcutaneous fat on the face, neck, trunk and abdomen with a lack of subcutaneous fat on the extremities. Triceps skinfold thickness was <5th percentile, even in the upper arm fat area. MRI of the tissue confirmed these findings, showing excessive and relatively symmetrical deposition of subcutaneous fat on the face, neck and upper trunk, with

disproportionate depletion of subcutaneous fat in the lower body (ESM Fig. 2). No other abnormalities related to FPLD were observed in this patient, and there was no acanthosis nigricans or hirsutism. The proband's mother, deceased at the age of 84, had a history of hypertriacylglycerolaemia and hypertension.

The proband's youngest sister was 50 years old at the time of investigation, with lipodystrophy, hypertriacylglycerolaemia and hypertension. She had no diabetes mellitus but fasting glucose (5.1 mmol/l) and insulin (138 pmol/l) levels suggested the presence of insulin resistance. Furthermore, the plasma glucose after an oral glucose load of 75 g was 8.3 mmol/l, indicating the presence of impaired fasting glucose.

The proband's son was 39 years old with prominent muscularity and accumulation of subcutaneous facial, neck and abdominal fat despite normal anthropometry. MRI of the tissue showed lipodystrophy at various anatomical sites (ESM Fig. 2). He also had a history of hypertriacylglycerolaemia and hypertension. He had no diagnosis of type 2 diabetes mellitus but fasting plasma glucose (4.9 mmol/l) and insulin levels (90 pmol/l) suggested the presence of insulin resistance.

We have no clinical data for the proband's nephew.

References

1. van Beekum O, Brenkman AB, Grontved L et al (2008) The adipogenic acetyltransferase Tip60 targets activation function 1 of peroxisome proliferator-activated receptor gamma. Endocrinology 149:1840–1849

2. Jeninga EH, van BO, van Dijk AD et al (2007) Impaired peroxisome proliferator-activated receptor gamma function through mutation of a conserved salt bridge (R425C) in familial partial lipodystrophy. Mol Endocrinol 21:1049–1065

3. Monajemi H, Zhang L, Li G et al (2007) Familial partial lipodystrophy phenotype resulting from a single-base mutation in deoxyribonucleic acid-binding domain of peroxisome proliferator-activated receptor-gamma. J Clin Endocrinol Metab 92:1606–1612